REGENERATION IN FUNDULUS AND ITS RELATION TO THE SIZE OF THE FISH.

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Przibram ('09) in his treatise on regeneration has reviewed the work bearing on the relation between the age of the animal and the ability to regenerate. The general conclusion is in the form of a law — " Die Regenerationsfahigkeit nimmt mit zunehmenden Alter eines Tierexemplares ab —." In the BIOLOGICAL BULLETIN ('07) the author came to the conclusion that the regeneration of the caudal fin of *Fundulus heteroclitus* was greater in the shorter than in the fishes of medium length and greater in these than in the longest fishes.

That conclusion was reached by comparing the average specific regeneration of the various groups. The specific regeneration is obtained by dividing the actual amount regenerated by the length of the animal, giving as a result the percentage amount regenerated by that specimen. This term was introduced by Zeleny. Now if we take any number and divide it successively by a series of numbers each greater than the preceding we will obtain a series of quotients each successively smaller and smaller. On referring to the experiments in his former paper the author found that the actual amounts of regeneration differed but little in the various specimens used, while the length of the animals increased, so that the case is as stated above — *i. e.*, that the greater specific regeneration in the shorter fishes is due largely to the fact that the divisor in case of short body length is smaller than the divisor in case of long body length, while the dividend (the actual amount regenerated) is about the same, so that naturally we should get smaller and smaller quotients, which in this case would be specific regeneration. This is seen when we refer to the former paper.

To test the matter further the writer repeated the experiment during the summer of 1908 using a larger number of fishes. The caudal fin of each specimen was removed at the same relative level on August 4 and the fish were placed in running sea-

Table I.

Fundulus heteroclitus.

	Length.	Amount Regenerated.	Specific Regeneration.		Length.	Amount Regenerated.	Specific Regeneration.
1	4.57 cm.	.57 cm.	.1247	55	6.80 cm.	.70 cm.	.1029
2	4.68	.54	.1154	56	6.81	.53	.0778
3	4.79	.58	.1210	57	6.86	.64	.0933
4	4.87	.66	.1355	58	6.89	.59	.0856
5	4.96	.65	.1311	59	6.90	.65	.0942
6	4.97	.60	.1201	60	6.94	.59	.0850
7	5.10	.54	.1058	61	6.96	.64	.0919
8	5.12	.59	.1152	62	6.97	.66	.0946
9	5.16	.58	.1124	63	7.10	.62	.0873
10	5.17	.54	.1044	64	7.12	.52	.0730
11	5.28	.60	.1117	65	7.24	.68	.0939
12	5.30	.61	.1151	66	7.26	.80	.1102
13	5.38	.64	.1187	67	7.29	.67	.0905
14	5.40	.60	.1111	68	7.29	.63	.0864
15	5.42	.54	.0996	69	7.38	.58	.0786
16	5.43	.64	.1178	70	7.39	.70	.0947
17	5.44	.61	.1122	71	7.40	.56	.0757
18	5.44	.53	.0974	72	7.44	.69	.0927
19	5.49	.65	.1184	73	7.45	.53	.0711
20	5.52	.69	.1250	74	7.48	.56	.0748
21	5.63	.66	.1172	75	7.49	.50	.0667
22	5.64	.61	.1081	76	7.50	.54	.0720
23	5.69	.59	.1031	77	7.50	.49	.0653
24	5.70	.78	.1368	78	7.52	.50	.0665
25	5.70	.66	.1158	79	7.53	.60	.0975
26	5.73	.68	.1193	80	7.56	.58	.0766
27	5.79	.60	.1036	81	7.60	.70	.0921
28	5.80	.58	.1000	82	7.62	.73	.0945
29	5.90	.60	.1002	83	7.69	.54	.0702
30	5.93	.65	.1096	84	7.74	.54	.0967
31	5.96	.57	.0956	85	7.77	.62	.0798
32	5.96	.64	.1074	86	7.87	.60	.0763
33	5.99	.78	.1302	87	7.88	.54	.0685
34	6.04	.67	.1109	88	7.92	.56	.0701
35	6.20	.62	.1000	89	7.94	.55	.0683
36	6.26	.67	.1070	90	8.00	.47	.0587
37	6.40	.70	.1094	91	8.00	.65	.0813
38	6.46	.50	.0774	92	8.07	.59	.0731
39	6.48	.68	.1049	93	8.10	.62	.0765
40	6.49	.52	.0801	94	8.23	.54	.0656
41	6.50	.60	.0923	95	8.23	.52	.0632
42	6.50	.60	.0908	96	8.25	.55	.0666
43	6.50	.57	.0877	97	8.27	.57	.0689
44	6.53	.53	.0812	98	8.27	.45	.0544
45	6.53	.54	.0827	99	8.37	.37	.0442
46	6.55	.63	.0962	100	8.38	.65	.0775
47	6.62	.47	.0710	101	8.38	.56	.0668
48	6.63	.60	.0815	102	8.39	.60	.0671
49	6.65	.63	.0947	103	8.40	.65	.0774
50	6.69	.63	.0942	104	8.56	.64	.0746
51	6.72	.54	.0803	105	8.69	.50	.0575
52	6.73	.63	.0937	106	8.91	.56	.0628
53	6.75	.42	.0622	107	9.00	.53	.0590
54	6.76	.64	.0974	108	9.73	.55	.0656

water in the Biological Laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass. I am indebted to Dr. Francis B. Sumner, the director of the laboratory, and to the commissioner, the Hon. George M. Bowers, for the facilities extended. The fishes were fed regularly until September 5, a period of a month, during which time new caudal fin tissue regenerated from the cut surface of each fin. The fishes were then removed, carefully preserved and later measured. There were alive, on September 5, 108 *Fundulus heteroclitus* and 50 *Fundulus majalis*. Table I. shows the length, the amount regenerated and the specific regeneration of each *Fundulus heteroclitus*. Following this is Table II. which shows the same. with regard to the *Fundulus majalis* used.

TABLE II.

Fundulus majalis.

	Length.	Amount Regenerated.	Specific Regeneration.		Length.	Amount Regenerated.	Specific Reneneration.
1	6.79 cm.	.60 cm.	.0899	26	8.08 cm.	.47 cm.	.0582
2	6.95	.58	.0835	27	8.13	.39	.0480
3	7.13	.53	.0743	28	8.18	.60	.0733
4	7.32	.54	.0738	29	8.20	.50	.0549
5	7.40	.55	.0743	30	8.23	.40	.0485
6	7.40	.45	.0601	31	8.24	.63	.0764
7	7.45	.55	.0768	32	8.26	.49	.0593
8	7.49	.55	.0734	33	8.26	.52	.0629
9	7.49	.55	.0734	34	8.33	.53	.0636
10	7.50	.49	.0658	35	8.45	.49	.0580
11	7.50	.55	.0733	36	8.50	.50	.0419
12	7.55	.48	.0635	37	8.57	.53	.0618
13	7.55	.40	.0530	38	8.60	.50	.0580
14	7.60	.53	.0698	39	8.60	.42	.0488
15	7.61	.55	.0722	40	8.67	.44	.0588
16	7.67	.53	.0691	41	8.72	.57	.0654
17	7.77	.55	.0708	42	8.75	.39	.0466
18	7.80	.55	.0705	43	8.80	.38	.0432
19	7.84	.35	.0446	44	8.96	.40	.0451
20	7.88	.50	.0634	45	8.91	.46	.0516
21	7.89	.45	.0570	46	9.17	.50	.0545
22	7.93	.59	.0744	47	9.20	.60	.0652
23	7.93	.55	.0693	48	9.60	.30	.0318
24	7.95	.56	.0704	49	9.73	.42	.0432
25	8.00	.40	.0500	50	10.50	.49	.0466

On examining the specific regeneration in the case of *Fundulus heteroclitus* there is seen to be a gradual fall in percentage from the shorter to the longer fishes. To make this more plain we can divide the fishes into groups ranging from the shorter to the

longer, differing in length from the adjacent groups by one half centimeter, find the average specific regeneration of each group and express the results as follows :

Group.	Range in Length.	Number of Specimens.	Average Specific Regeneration.
1	4.5– 5.0 cm.	6	.1243
2	5.0 5.5	13	.1108
3	5.5 6.0	14	.1123
4	6.0 6.5	7	.0885
5	6.5 7.0	22	.0878
6	7.0 7.5	13	.0843
7	7.5 8.0	14	.0762
8	8.0 8.5	14	.0672
9	8.5 9.0	3	.0649
10	9.0 9.5	1	.0590
11	9.5 10.0	1	.0656

From this arrangement it would appear that the shortest regenerated 12 per cent. of their length while the longest regenerated 6 + per cent. and that on the whole as the length of the fishes increases the percentage regeneration decreases. On the other hand if we run over the column giving the actual amounts of regeneration we see that in a general way they are much the same for the longer as well as for the shorter fishes. This becomes evident when we ascertain the average actual regeneration for each of the groups mentioned above. This can be arranged as follows :

Group	1	2	3	4	5	6	7	8	9	10	11
Av. Reg., cm.	.60	.59	.65	.62	.59	.62	.58	.56	.57	.53	.55

On examining again the table giving the actual regeneration of each specimen we find some that may be regarded as extreme variants. These are nos. 24 and 33, in which the regeneration is .78 cm., no. 66 with a regeneration of .80 cm. and no. 99 in which the regeneration is .37 cm.

If we make allowance for these and obtain new averages for the groups in which they occur our series will be as follows. We will disregard the last two groups on account of the small numbers of specimens.

Group	1	2	3	4	5	6	7	8	9
No. of Sp.	6	13	12	7	22	12	14	13	3
Av. Reg., cm.	.60	.59	.63	.62	.60	.60	.58	.57	.57

If we compare the average regeneration of groups 1 and 2, representing 19 fishes from 4.5 cm. to 5.5 cm. in length, with

the average regeneration of groups 8 and 9, representing 16 fishes from 8.0 cm. to 9.0 cm. in length, we find that there is a difference of but .025 cm. Again, if we compare the average regeneration of groups 2 and 3, representing 25 short fishes, with that of groups 7 and 8, representing 27 longer fishes, we find a difference of but .035 cm. The relation of the specific regeneration to the actual regeneration is represented by the following diagram (Fig. 1) in which the base line represents the length of the fishes — the upper curve *A–B* was formed by joining the points representing the specific regeneration in the various groups and therefore represents the relation of the specific regeneration to length. The lower curve, *C–D*, was formed by drawing a line through the points representing the actual regeneration in the various groups and therefore represents the relation of the actual regeneration to length. It is to be noted that while on the whole the curve representing relative specific regeneration falls, at the same time the curve representing the actual regeneration remains almost parallel with the base line although it will also be noted that there is an indication of a slight decrease in regeneration. There is a strong indication, however, that the longer fishes have regenerated almost as much tissue as the shorter in the same length of time.

But what is the condition in the case of the *Fundulus majalis* the results of the experiment with which are given in Table II. On arranging the results in a way similar to that used in the case of *Fundulus heteroclitus* we have the following:

Group.	Range in Length.	Number of Specimens.	Amount Regenerated.	Average Specific Regeneration.
1	6.5– 7.0 cm.	2	.59 cm.	.0867
2	7.0 7.5	7	.52	.0709
3	7.5 8.0	15	.51	.0658
4	8.0 8.5	11	.49	.0594
5	8.5 9.0	10	.46	.0521
6	9.0 9.5	2	.55	.0598
7	9.5 10.0	2	.36	.0373
8	10.0 10.5	1	.49	.0466

Here again it will be observed that there is a gradual decrease in the specific regeneration. The number of specimens in groups 1, 6, 7 and 8 is so small that too much value must not be placed on them. Although the total number of specimens is smaller in

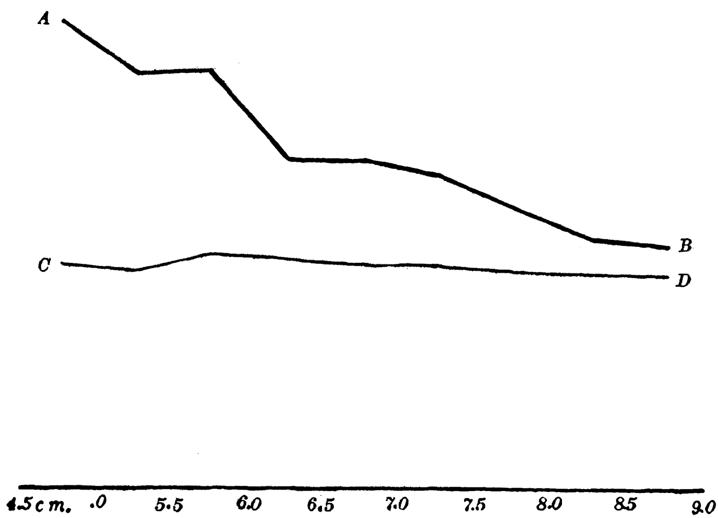

Fig. 1

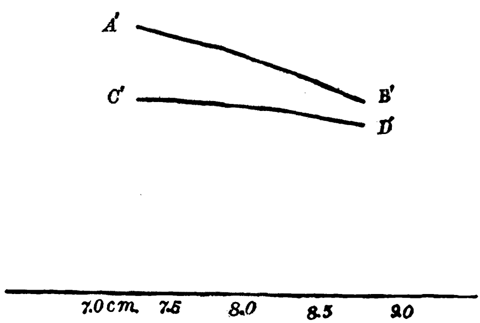

Fig. 2

this case, yet it is seen that the average regeneration is about the same, although here again is observed the slight decrease. The average regeneration of groups 2 and 3, consisting of 22 fishes between 7.0 and 8.0 cm. in length, is .515 cm., while that of groups 4 and 5, consisting of 21 fishes between 8.0 and 9.0 cm. in length, is .475 cm., showing a difference of .04 cm. between the two groups. These results are shown graphically in Fig. 2, in which the curve A'–B' shows the relation of the specific regeneration to length, while the curve C'–D' shows the relation of the actual amount regenerated to the length. Turning to the results recorded in the former paper we find that they are less satisfactory on account of the smaller numbers. But arranging the results recorded there in a manner similar to that used here we find that on the whole the longer fishes regenerate almost as much as the shorter, although the indication of the slight diminution of actual regeneration with age is not so clear, which may be due to the fact that there are less specimens in the various groups.

The general result seems to be then that the amount of regeneration in the period of time referred to appears to be about the same in fishes of all lengths, although there is an indication of a slight decrease in the case of increasing body length.

Professor Zeleny in a paper to be published shortly (Oct., '09) in the *Journal of Experimental Zoölogy* has found with respect to the regeneration of the tail of the salamander, *Amblystoma jeffersonianum* that rate of regeneration was as follows:

In a series	22.6	mm. long there was	a regeneration	of	.39	mm. per day.
"	26.5	"	"	"	.41	"
"	26.8	"	"	"	.39	"
"	51.6	"	"	"	.32	"
"	54.1	"	"	"	.27	"

These results agree with those described above. There is a maintenance of a high degree of regenerative power in the older specimens. There is also an indication of a slight decline such as we found in the case of the fishes used.

In carrying on an extensive series of observations on the age of fishes Fulton ('06) estimated the age by finding the different modal lengths that occurred in a large number of specimens of a given species. Though the numbers used in this experiment are

far too few to enable one to estimate the exact age of the various groups yet reasoning as Fulton did we can at least say that the longer fishes are the older. Stating the matter in terms of age the above experiments appear to indicate that within the limits of age as represented in the series the actual regeneration is the same or slightly decreases with age. It may be objected that the longer (or older) fishes regenerated more in mass than the smaller and that therefore should we determine the mass for each specimen we might find that the larger regenerated more than the smaller. To answer this objection let us suppose that Figs. 3 and 4 represent respectively the caudal fins of one of the shorter

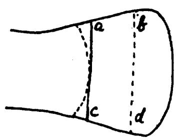

FIG. 3.

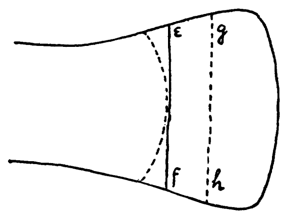

FIG. 4.

and one of the longer fishes. The straight vertical line in each case represents the place of amputation. The dotted vertical line represents the outer limit of new regenerating tissue at the end of a month. According to our results the perpendicular distance from the line *a–c* to the line *b–d* is nearly the same as that from *e–f* to *g–h*. When the amputation was made it left cells exposed along the surfaces represented by the lines *a–c* and *e–f*. In a short time the regeneration of new tissue began. In Wilson's "Cell," 00, page 388, we find that "measurements of cells from the epidermis, the kidney, the liver, the alimentary epithelium and other tissues show that they are on the whole as large in the dwarfs as in the giants. The body size depends on the total number of cells rather than on their size individually considered and the same appears to be the case in plants."

So we can conclude that the cells from which new tissue regenerates along the surface represented by the line *a–c* are of the same size as those represented by the line *e–f*. It is apparent that the tissue along the direction of *a–b* has been formed from cells at *a*, and that the tissue along the line *c–d* from cells at *c*.

So for every point in the line *a–c* the tissue opposite every point in this line has been formed from cells in the line *a–c* outward in a direction perpendicular to that line. And so for the newly formed tissue in case of Fig. 4, the tissue opposite every point in the line *e–f* has been formed from cells in the line *e–f* outward in a direction perpendicular to the line *e–f*. Of course in the case of larger fishes (Fig. 4) it is apparent that a greater mass of tissue is formed than is true of smaller fishes (Fig. 3). But is this not due to the fact that there are more cells along the surface represented by the line *e–f* from which regeneration can proceed than there are similar cells along the surface indicated by the line *a–c*. The solution of the problem as to the relation between age and rate of regeneration depends upon the results of measurements made in this manner. And by the amount of tissue regenerated has been and will be meant the length of newly formed tissue measured outward from the line of amputation.

If the cells in the shorter and longer (younger and older) fishes have the same degree of activity then the same amount of tissue ought to be formed outward in a line perpendicular to the cut surface in the same length of time. This is seen to be practically the condition in the case of the experiments presented here. What explanation can be offered for this? Jordan, '05, speaking of the growth of fishes says that "Most of them grow as long as they live and apparently live until they fall victims of some stronger species." Fulton, '06, gives the following law with regard to the growth of fishes: "Fishes approximately double their size and increase their weight about eight times after they have reached sexual maturity." Probably most of the fishes used in these experiments had reached sexual maturity. May we not then correlate this maintenance of a high degree of regenerative power with the continuous growth throughout the life of the members of this group.

But there appears to be a slight decrease in the amount regenerated as the age increases. In computing the difference of the means and the probable differences between adjacent groups of the different series it was found in most cases that at least twice the probable difference was less than the difference of the means and only in case of extreme groups was the difference of the

means less than three times the probable difference. This indicates that in adjacent groups the amount of regeneration is very nearly the same, but that on the whole there is a tendency toward a decrease, as we pass from the younger to the older. We can sum up our results in this statement: The power to regenerate new tissue remains remarkably active throughout life but as the fish grows older this power gradually diminishes, which after all is in agreement with Przibram's law. This also is in harmony with the view that regeneration is a growth phenomenon as shown above. Minot, '90, says: "There is a progressive loss of vitality going on probably throughout the entire period of life." Kellicott, '08, found that the organs of the dog-fish which have to do with nutrition and therefore the growth of the organism increase by constantly decreasing increments with increasing size of the animal. The slight decrease in regenerative power which we have noted above parallels then this slowly decreasing rate of growth characteristic of all animals, but which decrease is less evident in those forms having indeterminate growth such as fishes.

Kellicott, in the paper referred to above, found that while the organs of nutrition have increments of growth successively smaller, yet the "muscles and supporting tissues seem to outgrow the brain and viscera leading ultimately to a loss of physiological balance within the organism." But this decrease in growth of the organs of nutrition does not come on suddenly but gradually, so that it must eventually cause a gradual retardation of the growth of the entire animal. If this be true and if the rate of regeneration is affected by the rate of growth, then we should expect to find evidences of gradual diminution in the rate of regeneration. We have seen an indication of this in the above experiments. Finally, it has been noted that mammals and birds have little power of regeneration as compared with amphibia and fishes. May not this be possibly correlated with the different types of growth which these groups possess.

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